DSTL/JA100134

Aerobiology: Experimental considerations, observations and future tools

Allen E. Haddrell[1] and Richard J. Thomas[2]

School of Chemistry, Cantock's Close, University of Bristol, BS8 1TS[1]; Defence Science and Technology Laboratory (Dstl), Porton Down, Salisbury, Wiltshire, UK, SP4 0JQ[2]

Abstract

Understanding airborne survival and decay of microorganisms is important for a range of public health and biodefence applications including epidemiological and risk analysis modelling. Techniques for experimental aerosol generation, retention in aerosol phase and sampling require careful consideration and understanding so that they are representative of the conditions the bioaerosol would experience in the environment. This review explores current understanding of atmospheric transport in relation to advances and limitations of aerosol generation, maintenance in the aerosol phase and sampling techniques. Potential tools for the future are examined at the interface between atmospheric chemistry, aerosol physics and molecular microbiology that could explore heterogeneity and variability at the single droplet and single microorganism level within a bioaerosol. The review highlights the importance of method comparison and validation in bioaerosol research, and the benefits application of novel techniques could bring to increased understanding of aerobiological phenomena in diverse research fields, particularly during the progression of atmospheric transport where complex interdependent physicochemical and biological processes are occurring within bioaerosol particles.

INTRODUCTION

Aerosols injected into the atmosphere from the biosphere (bioaerosols) account for a significant portion of all atmospheric aerosols (1). Despite their low numbers relative to other natural aerosol, bioaerosols (whose sources include microorganisms contained within windblown dust and sea spray) are speculated to impact climate through behaving as efficient cloud condensation nuclei (2-3). Biological aerosols are also important from the perspective of human health being intimately involved in the transmission of many respiratory pathogens (4, 5).

Risk analysis modelling aims to develop predictive models of transmission and infection based on laboratory generation of aerosols containing respiratory pathogens. These experimental models are invaluable for understanding epidemic transmission, developing infection control measures and advising bioterror preparedness for public health (6-8). Effective risk modelling requires an in depth understanding of experimental aerosol techniques and their potential impact on the final outcome, whether that is aerosol decay, transmission rate or infectious dose.

This article reviews the current understanding, advances and limitations in laboratory aerobiological studies where the relationship between microorganism preparation, aerosol generation, evaporation, transport and fate cumulatively may affect the final outcome of inhalational infection or survival in the environment. In this review, the term “bioaerosol” will be limited to refer explicitly to infectious aerosol droplets containing living species, specifically bacteria and viruses; the study of this subset of bioaerosol comes with its own unique set of challenges that need to be recognized and addressed. The PubMed database was searched to identify relevant studies

using the strings: aerosol AND survival, bioaerosol AND generation, bioaerosol AND sampling. The terms bacteria and virus were interchanged for the term survival in the first search string; only published studies were included. References with no relation to bioaerosol as defined as 'infectious aerosol droplets' (e.g. fungal spores, pollen) were generally discarded unless the technology could be applied to the field. Retrieved studies were also reviewed for additional references. Although intrinsically linked to the general theme of this review, the development of inhalational animal models to replicate human disease is considered outside the scope and readers are directed to an extensive literature in this field (9-11).

AEROSOL GENERATION, SAMPLING AND POST-PROCESSING CONSIDERATIONS

Aerosol generation and sampling prior to microbiological analysis are conducted for a range of bioaerosol related research activities (e.g. determination of aerosol decay rates and inhalational infectious dose, efficacy of decontamination strategies, and evaluation of bioaerosol sampling technologies). These dynamic processes can cause damage due to shear forces acting on the microbial cells (12-27). Table 1 outlines some major aerosol generators and samplers used in aerobiological studies and the operating mechanisms. The majority of studies use reflux aerosol generators in conjunction with impingement to collect the generated aerosol. This system can be safely used in biocontainment laboratories for inhalational challenges and aerosol fate studies. However, comparative studies show that refluxing nebulizers produce the greatest loss of physiological function as a function of time in bacteria (16, 19-21, 24). The loss of function has been linked to membrane damage (13, 20, 24), release

of ions into media (e.g. PO_4^{2-}; 28), cell fragmentation (15, 23), reduction in ATP activity (29) and magnitude of associated electrical charge (30) as the bacteria remaining in the nebulizer repeatedly pass through the devices nozzle. Similar effects are observed for viruses (25). Repair of bacterial cells damaged by nebulization appears to be an energy dependent process with a requirement for divalent cations although independent of *de novo* RNA or protein synthesis (13, 31); it is unlikely that repair occurs in viruses due to their reliance on host cell factors for protein transcription and translation. In contrast, it has been reported that damage is reduced in non-refluxing aerosol generators where the microorganisms pass through the nozzle once (16, 24).

Sampling methods for airborne microorganisms include impingement, impaction, filtration, cyclonic separation, and electrostatic precipitation. This review will not cover all bioaerosol samplers, rather selecting the main sampling mechanisms and representative sampler models. The reader is directed to a couple of comprehensive reviews on bioaerosol sampling for further detail (32, 33). Each sampling technique has advantages and disadvantages for sampling microbial aerosols (Table 1) with the potential to cause microbial damage. Dependent on the microbe this damage may be transient: for example, impingement (AGI-30; 15 to 60 min) caused structural damage to *Pseudomonas fluorescens* cells with recovery achieved on non-selective media (15). Aerosol sampling times for determining infectious dose and aerosol decay rates generally range from 1-10 min which minimize the effects of microbial damage (22, 34). However, for infectious aerosols, few comparative studies of the bioefficiency of different sampling mechanisms. Where studies comparing samplers have been conducted, differences between microbial structures influence sampler bioefficiency; for example, infectivity and culturability differences were observed

between bacteriophages and influenza A virions sampled by the SKC biosampler and NIOSH cyclone (25, 35). Similar species dependent effects have been observed for bacteria in sampling bioefficiency; in particular *Bacillus* endospores tend to be less affected by aerosol sampling method (15, 17, 21, 22). One reason for differences in sampler bioefficiency is variation in sampling velocities that for impingement reaches 260 $m \cdot s^{-1}$, ten-fold greater than other samplers (36; Table 1). Secondly, the rapid rehydration that occurs during sampling can be detrimental to microorganisms (37-39).

Minimising stresses occurring during aerosol generation and sampling is hence critical to accurate representation of aerosol decay and infectivity. Aerosol generation stresses can be reduced by using single-pass devices that reduce the probability of a microorganisms being damaged (24). Depending on sampler choice, maximising recovery of microbes can be achieved in a number of ways. Prolonged sampling times is a consistent cause of reduced viability and hence collection times across all types of samplers and should be minimized (22, 40). The cell membrane is a major site of damage for Gram negative bacteria being aerosolised as sampled, demonstrated by increased sensitivity to hydrolytic enzymes (12). Impingement requires collection into liquid which can be optimised to reduce osmotic shock and maximise repair and recovery. For example, addition of compatible solutes and scavenging enzymes (i.e. trehalose, raffinose, polyhydric alcohols, betaine and catalase) can facilitate survival following the stresses associated with aerosol generation, transport and sampling (38, 41-46). Particle bounce and viability loss in impactors for vegetative *Bacillus subtilis* and *Escherichia coli* cells was reduced by applying a thin film of mineral oil significantly enhancing collection efficiency (47). Filtration methods provide high physical collection efficiencies, but bioefficiency can

be dependent on filtration time and post-processing procedures (21, 24, 48, 49). A major problem with filtration samplers is continued drawing of air through the filter desiccates collected microorganisms in a time-dependent manner. However, filtration onto gelatin membranes provides a medium that retains moisture and can be placed into warm media to recover collected microorganisms providing good bioefficiency (21, 24).

Post-sampling enumeration and storage are additional considerations. Enumeration can introduce error as organisms can be sensitive to impaction onto an agar surface (50), sensitive to the plating media (15) and the process of spread plating (51-53). Direct methods such as microscopy or flow cytometry in conjunction with various dyes or quantitative polymerase chain reaction (PCR) can indicate physiological activity of the collected microorganisms (15, 17, 54). Storage temperature, sampling solution and length of time can prompt microbial replication (or death) causing misrepresentation of the actual viability of the sampled bioaerosol (48). Samples should be processed as soon as possible after aerosol sampling; however this is highly dependent on the microorganism as for example, *Bacillus* endospores have been demonstrated to be less affected by storage temperature (4 and 25 °C) compared to *Escherichia coli*; however compared to immediate enumeration, both species had increased counts after extended periods of storage at 25 °C (10 and 24 h for *B. subtilis* and *E. coli* respectively) indicating significant disaggregation and/or multiplication in the collection medium, which in this case was sterile deionized water containing a small quantity of detergent (48).

The data indicates that the method of aerosol generation can damage the microorganism at the subcellular level, at the very least subtly, and influence resultant estimates of microbial viability in the aerosol phase. None of these

mechanisms are entirely representative of the natural transmission mechanisms of respiratory pathogens e.g. coughing and sneezing followed by deposition in the respiratory tract (4, 5). The complexity of fluid fragmentation and droplet formation of oro-respiratory secretions during coughs and sneezes has recently been elucidated with the viscoelastic properties of respiratory secretions playing a defining role in final droplet size (55, 56). Viscoelasticity of respiratory secretions will change with anatomical location (e.g. nasal, bronchial) and disease state (e.g. chronic bronchitis, sinusitis, cystic fibrosis) as a result of changes in mucin content which will also affect droplet sizes (57, 58). Natural aerosol transmission events are likely to be less violent than the aforementioned aerosol generation processes. Therefore, selection and validation of experimental regimes (aerosol generator, spray fluid composition and sampling) to minimize microbial damage, promote maximal recovery and most closely replicate the natural event being modelled, is important for interpretation of aerosol data used in risk analysis models. Based on this review, and more extensive reviews on sampling methodology (32, 33) it is apparent that given the variability in microorganisms responses to the stresses of aerosol generation and collection, then it is advisable to perform method validation for each particular microorganism. Testing a range of aerosol generators and samplers to ensure the behaviour of the microorganism within the system is understood facilitates appropriate selection of apparatus and methodology to maximise recovery during enumeration.

AEROSOL TRANSPORT AND PHYSICAL PROCESSING

The physicochemical properties of bioaerosol particles govern all of the biological processes within. The conditions in a bioaerosol particle that a microorganism will experience can be dramatically different than in bulk liquid; the solute concentrations commonly reach supersaturation (59), while the rate of water transport within the

droplet can vary by orders of magnitude (60). Both of these properties are regulated by total water present in the droplet. Thus a detailed understanding of the hygroscopic properties of a bioaerosol as a function of solute composition (including biological species itself) is critical for understanding and predicting longevity and overall infectivity.

The typical trajectory in RH for a respiratory pathogen would be high at the point of dispersion (>95%) to low during atmospheric transport (ambient relative humidity, RH) to high upon inhalation (>95%) (61). During its lifetime, the water activity (a_w) within a droplet equilibrates with the atmospheric RH through either the addition or removal of water (62). From droplets larger than 100 nm in size, the water activity is equal to the gas phase RH at equilibrium. The rate at which this mass flux occurs and the final particle size attained are a reflection of the temperature and humidity of the gas phase of the aerosol and the droplet solute (63, 64). Importantly, all microorganisms require water for activity as critical enzyme driven biochemical reactions (e.g. respiration). Interestingly, in studies looking at osmotic tolerance in bulk liquid phase, depending on bacterial species, multiplication and growth is inhibited at a_w values of 0.86 – 0.97 with further reductions inducing dormancy or eventually reducing viability (65, 66).

The hygroscopic behaviour of any multicomponent aerosol is dependent on the relative abundance of each chemical species in the solute, where each component will contribute a proportion to the uptake or loss of water (62). This paradigm holds true for bioaerosol, for example it has been shown that the solute concentration affects the hygroscopic growth of aerosolized *B. subtilis* and *Pseudomonas fluorescens* vegetative cells (67). However, to study the hygroscopic behaviour of aerosol where the aim is to generate predictive models, much information about the

solute is required. The relative abundance of each component within the aerosol is mandatory (68-72), as is a detailed understanding of how the various components within the solute interact with one another (73). While this is somewhat straight forward with regards to non-biological aerosol, it remains a major challenge in bioaerosols. For example, infected individuals coughing and sneezing will produce larger droplets with different concentrations of mucus and other organic and inorganic solutes compared to healthy individuals (58). Similarly, in laboratory studies, microbial culture conditions (liquid broth, solid agar and nutrient composition) and growth phase affect the concentration and types of nutrients present in the spray suspension and these factors influence aerosol survival (25, 74-78). Indeed, survival of a viral simulant, the bacteriophage MS2, differed in human derived saliva, artificial saliva and cell culture medium, with greatest decay observed in human derived saliva (79). This has been observed for other viruses and bacteria upon comparing survival after aerosolization from body fluids (natural or synthetic) and culture medium (80-83). This highlights the caution needed in extrapolation of results from the experimental to *in vivo* situations being modelled in risk analysis.

The primary challenge in experimental studies of the factors that regulate the hygroscopic behaviour of bioaerosol is to control and know the complete composition of the bioaerosol droplets. For example, a simple factor such as control of the number of organisms per droplet/particle is not trivial using conventional aerosolization processes. To attempt to address this specific issue in studies of laboratory generated bioaerosols, a particular size is selected for a nebulized and dried bioaerosol sample allowing estimation of the number of species per droplet prior to hygroscopic analysis (16). For more complex (and atmospherically relevant) bioaerosol, the hygroscopic behaviour of anthropogenic bioaerosol has been

estimated indirectly (84, 85). In these studies, the relative growth in bioaerosol particle size with increases in RH was estimated through correlation analysis between the temporal size distributions (aerodynamic diameter) of airborne fungi with meteorological information (RH).

Thermodynamic models to predict the hygroscopic behaviour of aerosol (e.g. Universal Quasichemical Functional Group Activity Coefficients; UNIFAC) have been used for bioaerosols to limited success (59, 86). Generally, these models are able to predict the hygroscopic behaviour of large and complex organic molecules through parameterization of the functional groups present (such as carboxylic acids; 87). Even though, organically, bioaerosol consists primarily of sugar alcohols and highly polar sugars (88), it remains unclear the extent to which these models can be used to predict the hygroscopic behaviour of bioaerosols (89). The reason for this is that even when the relative abundances of functional groups and chemical species within a single bioaerosol droplet are known, the accumulation of noncovalent interactions between these species is not; the presence of cellular membranes within the droplet could kinetically limit the hygroscopic behaviour of all the chemical species within the aerosol.

The limited number of comprehensive studies that explicitly study the physicochemical properties of bioaerosol is problematic. Their absence has constrained the means by which the longevity of suspended bioaerosol can be investigated.

DETERMINING BIOAEROSOL LONGEVITY

Bioaerosol longevity is simply the length of time in which a biological species will remain either infectious or viable while suspended as a single particle. In an ideal experiment, the entire composition of the target bioaerosols would be explored; as discussed in previously sections this is technically challenging due to the selectivity of samplers and the heterogeneity of bioaerosol composition. Despite this, numerous studies on bioaerosol longevity have been published.

Techniques for investigating survival of bioaerosols *in vitro* (Table 2) tend to either maintain the particles in the air column (i.e. 'dynamic bioaerosols') or captured on fine substrate such as spider silk or glue fibres (i.e. 'captured bioaerosols'). The rotating drum is probably the standard procedure used for aerosol longevity studies based on Goldberg and colleagues seminal design (90). Modifications have permitted greater control (e.g. *in situ* monitoring of parameters) and accessibility to a range of environmental parameters (e.g. temperature, UV, volatile organic compounds), and the suspension of larger aerosol particle sizes for sufficiently long periods of time (91-94). Methods based on capturing bioaerosols on microfibers derived from spider escape silk and glue gun fibres have been utilised with success (78, 95-97). Comparative studies on filoviruses have demonstrated that microthread captured bioaerosols decay at a similar rate as those held dynamically within rotating vessels (34, 98).

The methods for retention of microorganisms in the aerosol phase have been used extensively to determine biological decay in the airborne state as a function of time and a range of environmental conditions (Table 3). The aerosol is sampled at time intervals and the number of viable microorganisms enumerated enabling calculation of aerosol decay rate. Sampling method and subsequent microbiological processing and enumeration can alter the number of recovered microorganisms (15, 17, 21, 22).

Therefore it is important to minimize microbial stress during aerosol collection to facilitate accurate calculation of the decay rate. During method validation, it is important to differentiate biological decay from physical losses due to deposition on the walls of the vessel or removal from the microthreads due to turbulence (or the presence of antimicrobial substances on the silk). Physical loss in aerosol systems is determined by using physical tracers that will not biologically decay such as *Bacillus* spores, chemicals (e.g. fluorescein) or polymer beads (21, 99, 100). The decay rates of the target microorganism and the physical tracer can be compared and the true biological decay rate determined.

A disadvantage of these techniques is that they sample bulk aerosol and it is difficult to develop an appreciation of microenvironment heterogeneity occurring within individual aerosol droplets from the physicochemical and biological perspective. For example, each individual aerosol droplet is likely to have a different chemical composition, exacerbated by differences in particle size that manifest themselves biologically on the microorganisms incorporated within the droplets. Such differences may be a source of variability in how microbes respond and survive aerosol transport.

ENVIRONMENTAL FACTORS AFFECTING MICROBIAL LONGEVITY DURING ATMOSPHERIC TRANSPORT AND BACTERIAL SURVIVAL MECHANISMS

A large number of environmental and meteorological factors can influence microbial survival during aerosol transport (Table 3), and to provide greater context for interpretation of results the environmental features of the sampling site should be described. The fate of the microorganism is likely dictated by its physiological status

294 which is a combinatorial consequence of the atomisation process (e.g. spray device,
295 cough, sneeze) with the associated evaporative stresses of aerosol transport and
296 rehydration during inhalation (or sampling into liquid). The mechanisms by which the
297 microorganisms perish have been partially elucidated and depend on the
298 composition of the droplet and surrounding atmosphere.

299 Atmospheric oxidants (e.g. reactive oxygen and nitrogen species, sulphur dioxide,
300 ozone) will impact on microbial longevity by either directly acting on the organism or
301 with constituents within the aerosol droplet (101, 102). Presence of oxygen has been
302 demonstrated to have a deleterious effect on airborne coliform bacteria, particularly
303 at RH less than 40%, and hypothesised to be due to production of reactive oxygen
304 species (ROS) by Maillard reactions (31, 103). Maillard reactions are amino-carbonyl
305 reactions occurring between amino groups on proteins and reducing sugars that
306 cause oxidation of macromolecules and death in microorganisms (104). In airborne
307 microorganisms, these reactions may be the cause of oxidative damage to critical
308 enzymes (44, 105-107), phospholipids and nucleic acids causing metabolic
309 imbalance, destabilisation of membranes and reducing repair activity (31).
310 Interestingly, recently Maillard chemistry has been implicated as a source of organic
311 compounds within atmospheric aerosols altering particle viscosity and hence the
312 diffusivity rate of water and reactive gases (108). Bioaerosols (including virus,
313 vegetative bacteria, spores and peptides) subjected to atmospheric ozone
314 concentrations and variations in RH showed temporal changes in fluorescence
315 spectra related to oxidation and hydrolysis of tryptophan (109-111). Although
316 survival is generally greater at higher RH (>80%), certain values (i.e. 70-85% RH for
317 *E. coli* B; 41, 44) produce a large decrease in aerosol survival (41, 107, 112, 113).
318 Likewise, RH dependent changes in salt concentrations and pH within droplets

influence virus viability causing conformational changes in surface proteins and membrane fluidity affecting infectivity (114).

Solar irradiation and atmospheric pollutant gases (including open air factor; OAF) are two further environmental parameters that can significantly affect longevity in the aerosol phase. Solar irradiation markedly decreased viability compared to control conditions that simulate the night (46, 78, 115-118). Particle size-dependent survival against solar irradiation has been observed with bacterial clusters persisting for longer periods (78, 117). Terrestrial solar spectral irradiance varies through the day, with season and with geographical location (119). The UV wavelengths are of most importance for inactivating microorganisms (116, 117), where UV-A and UV-B reach the troposphere with the potential to cause a variety of DNA genomic lesions and damage to nucleic acids, proteins and lipids due to generation of reactive oxygen species (120-121). It is important that studies using both simulated and natural solar irradiation report variables such as solar intensity as accurately as is reasonably possible to facilitate data interpretation and standardisation between laboratories.

Atmospheric constituents such as various pollutant gases and secondary organic aerosols (SOAs; Table 3) have been demonstrated to have significant deleterious effects on aerosol longevity (31, 93, 122, 123-130). Many of these may contribute to a phenomenon known as ‘open air factor’ (OAF) where aerosolized microorganisms exposed to open climatic conditions decay more rapidly than those in enclosed laboratory vessels subjected to similar temperature and RH (31, 123-125, 129, 130). The precise nature of OAF is not fully understood but is hypothesised to involve a number of highly reactive products (e.g. hydroxyl radicals) from photochemical interactions between ozone and unsaturated hydrocarbons from anthropogenic (e.g. engine-related alkenes) and non-anthropogenic sources (e.g. plant turpenes) (31,

123). The reactive species rapidly oxidise and degrade macromolecules such as lipids, proteins and nucleic acids (31, 131). The effect of OAF is enhanced at high humidity (80-90% RH) for both *E. coli* and *Micrococcus albus* (123). Such humidity effects warrant investigation, possibly relating to the increased water content of aerosol particles at higher humidity.

How microbes regulate and survive aerosol transport is undetermined. Evidence suggests that the ability for transcription and translation to occur in the environment of an evaporating droplet is reduced (31, 132, 133). Evaporation and rehydration of aerosol particles imparts osmotic and desiccative stress on the microbe reflective of the humidity of the surrounding atmosphere and composition of the particle. The molecular response of many bacterial species to osmotic stress and desiccation is well documented from research understanding survival in food matrices, aquatic and marine systems and terrestrial environments (66). Hyperosmotic stress (i.e. increased a_w) causes a reduction in cytoplasmic volume as water exits the bacterium; concomitantly cell growth and respiration cease as the bacterium adapts to the hyperosmotic conditions. Initially charged solutes (e.g. K^+ ions, glutamate) are accumulated via specific uptake mechanisms (66, 134-136). Interestingly, inability to control efflux of K^+ ions correlated with decreased survival in aerosolised *E. coli* cells (28, 137). Synthesis of compatible solutes (e.g. trehalose) or uptake from the surrounding media (e.g. glycine betaine, proline) stabilises proteins, enzymes and membrane phospholipids enable critical biochemical processes to continue in hyperosmotic stressed bacteria. As the bacterial cell stabilises, a number of proteins are synthesised prompting repair of DNA damage, scavenging of reactive oxygen species and degradation of misfolded proteins (66, 134-136). Osmotically adapted cells often show cross-tolerance to other stresses such as high temperature and

oxidative shock (138). Recently, *E. coli* subjected to rapid downshift in a_w (0.993 to 0.960) in media was demonstrated to control protein misfolding by transient expression of the RpoE and RpoH regulons in conjunction with the RpoS regulon to facilitate prolonged adaptation to the hyperosmotic conditions (139).

The molecular studies described above have all been conducted in bulk solution phase and expose the microorganisms to hyperosmotic stress. Microorganisms will be exposed to hyperosmotic conditions within an evaporating droplet (i.e. low a_w conditions), enabling speculation that similar molecular mechanisms play role in bacterial survival within evaporating aerosol droplets. As will be discussed later, advances in atmospheric chemistry and single cell genomic techniques means that investigation of whether similar molecular mechanisms occur in an aerosol droplet as a function of evaporation rate and droplet composition are on the horizon. Importantly, if airborne microorganisms can induce adaptive responses promoting survival then there is the potential that colonisation and infection of the respiratory tract is primed whilst the bacteria are transported in the atmosphere. Any induced virulence factors would offer attractive targets for combating respiratory infection.

NEW TECHNIQUES FOR ADVANCING AEROSOL SCIENCE AND AEROBIOLOGY

Bioaerosols, even when produced under controlled laboratory conditions, are complex. They are generally polydisperse in terms of both physicochemical and biological properties, and the heterogeneity in the nature of the bioaerosol evolves with time and distance from the source. Technological advances in the fields of aerosol science and molecular biology are timely to facilitate multidisciplinary

393 approaches to understand heterogeneity at the single droplet and single
394 microorganism level (including microbial aggregates) and to explore the
395 fundamentals of biological decay and survival in aerosol droplets.

396 Optical techniques such as optical tweezers and electrodynamic balances where
397 single aerosol droplets can be captured and levitated within an electric field for
398 periods of time (seconds to days) have been extensively used in atmospheric
399 chemistry to investigate heterogeneous chemistry, phase separation, hygroscopicity
400 and ice nucleation activity using analytical techniques including Raman
401 microspectroscopy (140-145). Utilisation of these techniques for biological aerosol
402 has been limited to date. However, optically trapped single biological cells in solution
403 produce characteristic Raman scattering signatures (146-149) and *E. coli* exposed to
404 1-butanol resulted in spectroscopic and anisotropic detection of real-time phenotypic
405 changes in fatty acid composition and membrane fluidity (149). Although these
406 studies were conducted in liquid bulk solution rather than aerosol droplets, it
407 exemplifies the power of the technology. Furthermore, such techniques are being
408 used to explore individual aerosol particles containing microorganisms, fungal spores
409 and pollen (150-152). The electrodynamic balance technique has been used to
410 accurately deposit single particles containing respiratory syncytial virus onto airway
411 epithelial cells enabling the cellular response to infection to be analysed (153). This
412 technique enables interaction at the air-cell interface with single aerosol particles, a
413 more representative scenario than the air-liquid interface studies commonly
414 conducted for *in vitro* infection studies. It is a technique that seems applicable
415 although currently rarely applied to understanding the heterogeneity of bioaerosols at
416 the single droplet and microorganism level.

Microbial cells respond to environmental stimuli by regulating gene expression resulting in modulation of the quantities and composition of functional proteins available to combat a particular stressful condition. Transcriptional analysis and insertional mutagenesis have been used to identify bacterial genes regulated in response to stresses associated with aerosol survival such as desiccation, and osmotic pressure (136, 154). Currently, these techniques have not been applied to aerosolised microbial populations, however it can be hypothesised that similar responses may be expected and warrant exploration. The relative abundance of particular proteins critical to aerosol survival will vary from cell to cell. Exploring this heterogeneity at the single cell level is complicated due to the relatively low abundance of stress-responsive proteins. However, the last five years have seen significant advances in molecular techniques enabling exploration of the genomic, proteomic or 155-158). Techniques for isolating single cells such as flow cytometry and microfluidics can be combined with techniques such as PCR and next-generation sequencing for probing the transcriptional response of single cells (159). Indeed, single cell genomic techniques have been applied to understanding airborne metagenomes in urban settings (160, 161). Application to aerosolised populations in a laboratory setting would seem straightforward. However, care in experimental design would be needed to discriminate the true effects of aerosol transport and the stresses of aerosol generation and sampling.

These emerging technologies have the potential to dramatically impact numerous areas of bioaerosol science. They will lead to improved parameterization of the fundamental properties of bioaerosol, such as the interplay between environmental conditions with species longevity and/or gene expression. This data will lead to better predictions of disease dynamics in areas such as general industrial hygiene,

animal husbandry, hospital design and biosecurity. Furthermore, the data collected from these laboratory based instruments will inform conventional research of environmental samples.

CONCLUDING REMARKS

Experimental factors affect the microbiological sample taken forward for quantification of infectious dose or biological decay rate. Therefore a thorough understanding of the sampling and enumeration process is critical to interpretation of the final data set. Furthermore, no single aerosol generation or sampling method is likely to suit all purposes (i.e. size selectivity, species sensitivity), therefore the experimental apparatus should be selected based on the hypothesis and microorganism being tested and the data interpreted alongside the caveats associated with the methodology. For experiments designed to generate data for input into risk analysis determination of human inhalational exposure then it is recommended that aerosol generators, samplers (and collection fluid) be used that cause minimal damage or promote maximal recovery of the microorganisms during collection to prevent underestimation of risk estimates.

Fundamental questions remain regarding aerosol transmission of respiratory pathogens, particularly the underlying mechanisms of survival and/or death during aerosol transport and the role the microenvironment of the droplet plays as it evaporates then rehydrates during inhalation. However, as outlined in this review, advances in distinct scientific fields could support a systematic dissection of the biological response of microorganisms within compositionally controlled aerosol droplets within specific atmospheric conditions. It is envisaged that within the next

ten years multidisciplinary approaches combining existing and novel techniques in atmospheric chemistry, aerobiology and molecular biology will converge and begin to dissect and empirically understand the mechanisms of microorganisms survival and decay in the aerosol state and the effect on infectivity and disease transmission.

ACKNOWLEDGEMENTS

Dr Allen Haddrell and Dr Richard Thomas recognise the funding support of Elizabeth Blackwell Institute for Health Research and the Ministry of Defence respectively. Thanks are extended to Prof Jonathon Reid for critical review of the manuscript.

REFERENCES

1. Jaenicke, R. 2005. Abundance of cellular material and proteins in the atmosphere. *Science* 308:73-73.
2. Sesartic A, Lohmann U, Storelvmo T. 2013. Modelling the impact of fungal spore ice nuclei on clouds and precipitation. Environ Res Lett 8:doi:10.1088/1748-9326/8/1/014029.
3. Konstantinidis KT. 2014. Do airborne microbes matter for atmospheric chemistry and cloud formation? Environ Microbiol 16:1482-1484.
4. Gralton J, Tovey E, McLaws M-L, Rawlinson WD. 2011. The role of particle size in aerosolised pathogen transmission: A review. J Infect 62:1-13.
5. Fernstrom A, Goldblatt M. 2013. Aerobiology and its role in transmission of infectious diseases. J Pathog 2013:1-13. http://dx.doi.org/10.1155/2013/493960.
6. Lander W, van Kerckhove K, Chao DL, Hens N, Beutels P. 2012. A nice day for an infection? Weather conditions and social contact patterns relevant to influenza transmission. PLoS One 7:e48695.
7. Hong T, Gurian PL, Huang Y. Haas CN. 2012. Prioritizing risks and uncertainties from intentional release of selected Category A pathogens. PLoS One 7:e32732.

8. Milton DK. 2012. What was the primary mode of smallpox transmission? Implications for biodefense. Front Cell Infect Microbiol 2:1-7.
9. Kukavica-lj I, Levesque RC. 2008. Animal models of chronic lung infection in *Pseudomonas aeruginosa*: useful tools for cystic fibrosis studies. Lab Anim 42:389-412.
10. Thangavel RR, Bouvier NM. 2004. Animal models for influenza virus pathogenesis, transmission and immunology. J Immunol Meth 410:60-79.
11. Swearengen JR. 2012. *In* Biodefense Research Methodology and Animal Models. CRC Press.
12. Hambleton P. 1970. The sensitivity of Gram-negative bacteria, recovered from aerosols, to lysozyme and other hydrolytic enzymes. J Gen Microbiol 61:197-204.
13. Hambleton P. 1971. Repair of wall damage in *Escherichia coli* recovered from an aerosol. J Gen Microbiol 69:81-88.
14. Kang Y-J, Frank JF. 1989. Biological aerosols: a review of airborne contamination and its measurement in dairy processing plants. J Food Prot 52:512-524.
15. Terzieva S, Donnelly J, Ulevicius V, Grinshpun S, Willeke K, Stelma GN & Brenner KP. 1996. Comparison of methods for detection and enumeration of airborne microorganisms collected by liquid impingement. Appl Environ Microbiol 62:2264-2272.
16. Reponen T, Willeke K, Ulevicius V, Grinshpun SA, Donnelly J. 1997. Techniques for dispersion of microorganisms into air. Aero Sci Technol 27:405-421.
17. Rule AM, Kesavan J, Schwab KJ, Buckley TJ. 2007. Application of flow cytometry for the assessment of preservation and recovery efficiency of bioaerosols samplers spiked with *Pantoea agglomerans*. Environ Sci Technol 41:2467-2472.

18. Hermann JR, Zimmerman JJ. 2008. Analytical sensitivity of air samplers based on uniform point-source exposure to airborne Porcine reproductive and respiratory syndrome virus and swine influenza virus. Can J Vet Res 72:440-443.
19. Eninger RM, Hogan CJ, Biswas P, Adhikari A, Reponen T, Grinshpun SA. 2009. Electrospray versus nebulization for aerosolization and filter testing with bacteriophage particles. Aero Sci Technol 43:298-304.
20. Thomas RJ, Webber D, Hopkins R, Frost A, Laws T, Jayasekera P, Atkins T. 2011. The cell membrane as a major site of damage during aerosolization of *Escherichia coli*. Appl Environ Microbiol 77:920-925.
21. Dabisch P, Yeager J, Kline J, Klinedinst K, Welsch A, Pitt ML. 2012a. Comparison of the efficiency of sampling devices for aerosolized *Burkholderia pseudomallei*. Inhal Toxicol 24:247-254.
22. Dabisch P, Yeager J, Kline J, Klinedinst K, Welsch A, Pitt ML. 2012b. Recovery efficiencies for *Burkholderia thailandensis* from various aerosol sampling media. Front Cell Infect Microbiol 2:1-7.
23. Zhen H, Han T, Fennell DE, Mainelis G. 2013. Release of free DNA by membrane-impaired bacterial aerosols due to aerosolization and air sampling. Appl Environ Microbiol 79:7780-7789.
24. Zhen H, Han T, Fennell DE, Mainelis G. 2014. A systemic comparison of four bioaerosols generators: affect on culturability and cell membrane integrity when aerosolizing *Escherichia coli* bacteria. J Aero Sci 70:67-79.
25. Turgeon N, Toulouse M-J, Martel B, Moineau S, Duchaine C. 2014. Comparison of five bacteriophages as models for viral aerosol studies. Appl Environ Microbiol 80:4242-4250.

26. Fennelly KP, Tribby MD, Wu CY, Weil GL, Radonovich LJ, Loeb JC, Lednicky JA. 2015. Collection and measurement of aerosols of viable influenza virus in liquid media in an Andersen cascade impactor. Virus Adapt Treat 7:1-9.
27. Han T, Wren M, DuBois K, Therkom J, Mainelis G. 2015. Application of ATP-based bioluminescence for bioaerosol quantification: effect of sampling method. J Aerosol Sci 90:114-123.
28. Anderson JD, Dark FA. 1967. Effects of aerosolization upon survival and potassium retention by various bacteria. J Gen Microbiol 52:99-105.
29. Han T, Wren M, DuBois K, Therkorn J, Mainelis G. 2015. Development of ATP Bioluminescence method for rapid bioaerosol quantification. J Aero Sci 90:114-123,
30. Mainelis G, Willeke K, Baron P, Reponen T, Grinshpun SA, Gorny R, Trakumas S. 2001. Electrical charges on airborne microorganisms. Aero Sci 32:1087-1110.
31. Cox CS. 1987. The aerobiological pathway of microorganisms. John-Wiley & Sons, New York.
32. Kesavan J, Sagripanti J-L. 2015. Evaluation criteria for bioaerosol samplers. Environ Sci Process Impact doi:10.1039/c4em00510d
33. Grinshpun S, Buttner M, Mainelis G, Willeke K. 2016. Sampling for airborne microorganisms. In Yates, M, Nakatsu, C, Miller, R, Pillai, S (ed), Manual of Environmental Microbiology, 4th ed. ASM Press, Washington, DC
34. Piercy TJ, Smither SJ, Steward JA, Eastaugh L, Lever MS. 2010. The survival of filoviruses in liquids, on solid substrates and in a dynamic aerosol. Appl Microbiol 109:1531-1539.

562 35. Cao G, Noti JD, Blachere FM, Lindsley WG, Beezhold DH. 2011. Development of an
563 improved methodology to detect infectious airborne influenza virus using the NIOSH
564 bioaerosol sampler. J Environ Monit 13:3321:3328.
565 36. Mainelis G, Grinshpun SA, Willeke K, Reponen T, Ulevicius V, Hintz PJ. 1999.
566 Collection of airborne microorganisms by electrostatic precipitation. Aero Sci
567 Technol 30:127-144.
568 37. Benbough JE. 1971. Some factors affecting the survival of airborne viruses. J Gen
569 Virol 10:209-220.
570 38. Marthi B. 1994. Resuscitation of microbial aerosols. p 192-225. *In* Lighthart B, Mohr
571 AJ (eds), Atmospheric microbial aerosols: theory and applications. Springer Science,
572 New York.
573 39. Lang E, Zoz F, Iaconelli C, Guyot S, Alvarez-Martin P, Beney L, Perrier-Cornet J-M,
574 Gervais P. 2016. Recovery estimation of dried foodborne pathogens is directly
575 related to rehydration kinetics. PLoS One 11:e0160844.
576 40. Juozaitis A, Willeke K, Grinshpun SA, Donnelly J. 1994. Impaction onto a glass slide
577 or agar versus impingement into a liquid for the collection and recovery of airborne
578 microorganisms. Appl Environ Microbiol 60:861–870.
579 41. Cox CS. 1966a. The survival of *Escherichia coli* sprayed into air and into in nitrogen
580 from distilled water and from solutions of protecting agents, as a function of relative
581 humidity. J Gen Microbiol 43:383-399.
582 42. Marthi B, Lighthart B. 1990. Effects of betaine on enumeration of airborne bacteria.
583 Appl Environ Microbiol 56:1286-1289.
584 43. Marthi B, Fieland VP, Walter M, Seidler RJ. 1991. Resuscitation effects of catalase
585 on airborne bacteria. Appl Environ Microbiol 57:2775-2776.

586 44. Webb, SJ. 1969. The effects of oxygen on the possible repair of dehydration
587 damage by *Escherichia coli*. J Gen Microbiol 58:317-326.
588 45. Cox CS. 1968a. The aerosol survival of *Escherichia coli* B in nitrogen, argon and
589 helium atmospheres and the influence of relative humidity. J Gen Microbiol 50:139-
590 147.
591 46. Webb SJ, Tai CC. 1968. Lethal and mutagenic action of 3200-4000 Å light. Can J
592 Microbiol 14:727-735.
593 47. Xu Z, Wei K, Wu Y, Shen F, Chen Q, Li M, Yao M. 2013. Enhancing bioaerosol
594 sampling by Andersen impactors using mineral-oil-spread agar plate. PLOS One
595 8:e56896
596 48. Li C-S, Lin Y-C. 2001. Storage effects on bacterial concentration: determination of
597 impinger and filter samples. Sci Total Environ 278:231-237.
598 49. Dybwad M, Skogan G, Blatny JM. 20154. Comparative testing and evaluation of nine
599 different air samplers: end-to-end sampling efficiencies as specific performance
600 measurements for bioaerosols applications. Aero Sci Technol 48:282-295.
601 50. Stewart SL, Grinshpun SA, Willeke K, Terzieva S, Ulevicius V & Donnelly J. 1995.
602 Effect of impact stress on microbial recovery on an agar surface. Appl Environ
603 Microbiol 61:1232-1239.
604 51. Thomas P, Sekhar AC, Mujawar MM. 2012. Nonrecovery of varying proportions of
605 viable bacteria during spread plating governed by the extent of spreader usage and
606 proposal for an alternate spotting-spreading approach to maximize the CFU. J Appl
607 Microbiol 113:139-150.

608 52. Thomas P, Mujawar MM, Sekhar AC & Upreti R. 2014a. Physical impaction injury
609 effects on bacterial cells during spread plating influenced by cell characteristics of
610 the organisms. J Appl Microbiol 116:911-922.
611 53. Thomas P, Sekhar AC, Mujawar MM. 2014b. Vulnerability of *Bacillus* spores and of
612 related genera to physical impaction injury with particular reference to spread-
613 plating. J Appl Microbiol 117:1358-1372.
614 54. Allegra S, Leclerc L, Massard PA, Girardot F, Riffard S, Pourchez S. 2016.
615 Characterization of aerosols containing *Legionella* generated upon nebulization. Sci
616 Rep doi:10.1038/srep33998
617 55. Bourouiba L, Dehandschoewercker E, Bush JWM. 2014. Violent expiratory events:
618 on coughing and sneezing. J Fluid Mech 745:537-563.
619 56. Scharfman BE, Techet AH, Bush JWM, Bourouiba L. 2016. Visualization of sneeze
620 ejecta: steps of fluid fragmentation leading to respiratory droplets. Exp Fluids
621 57:doi:10.10007/s00348-015-2078-4.
622 57. Lai SK, Wang Y-Y, Wirtz D, Hanes J. 2009. Micro- and macrorheology of mucus.
623 Adv Drug Deliv Rev 61:86-100.
624 58. Hersen G, Moularat S, Robine E, Ghin E, Corbet S, Vabret A. 2008. Impact of health
625 on particle size of exhaled respiratory aerosols: case-control study. Clean 36:572-
626 577.
627 59. Clegg SL, Seinfeld JH, Brimblecombe P. 2001. Thermodynamic modelling of
628 aqueous aerosols containing electrolytes and dissolved organic compounds. J
629 Aerosol Sci 32:713-738.
630 60. Power RM, Simpson SH, Reid JP, Hudson AJ. 2013. The transition from liquid to
631 solid-like behaviour in ultrahigh viscosity aerosol particles. Chem Sci 4:2597-2604.

632 61. Elad D, Wolf M, Keck T. 2008. Air-conditioning in the human nasal cavity. Resp
633 Physiol Neurobiol 163:121-127.
634 62. Clegg SL, Seinfeld JH, Edney EO. 2003. Thermodynamic modelling of aqueous
635 aerosols containing electrolytes and dissolved organic compounds. II. An extended
636 Zdanovskii-Stokes-Robinson approach. J Aero Sci 34:667-690.
637 63. Davies JF, Haddrell AE, Rickards AMJ, Reid JP. 2013. Simultaneous Analysis of the
638 Equilibrium Hygroscopicity and Water Transport Kinetics of Liquid Aerosol. Anal
639 Chem 85:5819–5826.
640 64. Davies JF, Miles REH, Haddrell AE, Reid JP. 2014. Temperature dependence of the
641 vapor pressure and evaporation coefficient of supercooled water, J Geophys Res -
642 Atmos 119:931–940.
643 65. Potts M. 1994. Desiccation tolerance of prokaryotes. Microbiol Rev 58:755-805.
644 66. Burgess CM, Gianotti A, Gruzdev N, Holah J, Kochel S, Lehner A, Margas E,
645 Schmitz Esser S, Sela S, Tresse O. 2016. The response of foodborne pathogens to
646 osmotic and desiccation stresses in the food chain. Int J Food Microbiol 221:37-53.
647 67. Johnson DL, Pearce TA, Esmen NA. 1999. The effect of phosphate buffer on
648 aerosol size distribution of nebulized *Bacillus subtilis* and *Pseudomonas fluorescens*
649 bacteria. Aero Sci Tech 30:202-210.
650 68. Carslaw K S, Clegg SL, Brimblecombe P. 1995. A thermodynamic model of the
651 system HCl-HNO_3-H_2SO_4-H_2O, including solubilities of HBr, from less than 200 to
652 328 K. J Phys Chem 99:11557-11574.
653 69. Clegg SL, Brimblecombe P. 1995a. A generalized multicomponent thermodynamic
654 model applied to the $(NH_4)_2SO_4$-H_2SO4-H_2O system to high supersaturation and low
655 relative humidity at 298.15 K. J Aero Sci 26:19-38.

70. Clegg SL, Brimblecombe P. 1995b. Application of a multicomponent thermodynamic model to activities and thermal properties of 0-40 mol Kg^{-1} aqueous sulfuric acid from less than 200-K to 328-K. J Chem Eng Data 40:43-64.
71. Clegg SL, Ho SS, Chan CK, Brimblecombe P. 1995. Thermodynamic properties of aqueous $(NH_4)_2SO_4$ to high supersaturation as a function of Ttemperature. J Chem Eng Data 40:1079-1090.
72. Bowman FM, Melton JA. 2004. Effect of activity coefficient models on predictions of secondary organic aerosol partitioning. J Aerosol Sci 35:1415-1438.
73. Haddrell AE, Hargreaves G, Davies JF, Reid JP. 2013. Control over hygroscopic growth of saline aqueous aerosol using Pluronic polymer additives. Int J Pharm 443:183-192.
74. Brown AD. 1953. The survival of airborne microorganisms II. Experiments with *Escherichia coli* near 0°C. Aust J Biol Sci 6:470-480.
75. Won WD, Ross, H. 1966. Effect of diluent and relative humidity on apparent viability of airborne *Pasteurella pestis*. Appl Microbiol 14:742-745.
76. Cox CS. 1971. Aerosol survival of *Pasteurella tularensis* disseminated from the wet and dry states. Appl Microbiol 21:482-486.
77. Faith SA, Smith LP, Swatland AS, Reed DS. 2012. Growth conditions and environmental factors impact aerosolization but not virulence of *Francisella tularensis* infection in mice. Front Cell Infect Microbiol 2:1-10. doi: 10.3389/fcimb.2012.00126.
78. Handley BA, Webster AJF. 1995. Some factors affecting the airborne survival of bacteria outdoors. J Appl Bacteriol 79:368-378.

79. Zuo Z, Kuehn TH, Bekele AZ, Mor SK, Verma H, Goyal SM, Raynor PC. 2014. Survival of airborne MS2 bacteriophage generated from human saliva, artificial saliva, and cell culture medium. Appl Environ Microbiol 80:2796-2803.

80. Ijaz MK, Sattar SA, Johnson-Lussenburg CM, Springthorpe VS, Nair RC. 1985. Effect of relative humidity, atmospheric temperature, and suspending medium on the airborne survival of human rotavirus. Can J Microbiol 31:681-685.

81. Lever MS, Williams A, Bennett AM. 2000. Survival of mycobacterial species in aerosols generated from artificial saliva. Lett Appl Microbiol 31:238-241.

82. Donaldson AI. 1972. The influence of relative humidity on the aerosol stability of different strains of foot-and-mouth disease virus suspended in saliva. J Gen Virol 15:25-33.

83. Barlow DF, Donaldson AI.1973. Comparison of the aerosol stabilities of foot-and-mouth disease virus suspended in cell culture fluid or natural fluids. J Gen Virol 20:311-318.

84. Liao CM, Luo WC, Chen SC, Chen JW, Liang HM. 2004. Temporal/seasonal variations of size-dependent airborne fungi indoor/outdoor relationships for a wind-induced naturally ventilated airspace. Atmos Environ 38:4415-4419.

85. Liao CM, Luo WC. 2005. Use of temporal/seasonal- and size-dependent bioaerosol data to characterize the contribution of outdoor fungi to residential exposures. Sci Total Environ 347:78-97.

86. Fredenslund A, Jones RL, Prausnitz JM. 1975. Group-contribution estimation of activity coefficients in nonideal liquid mixtures. AIChE J 21:1086–1099.

87. Peng C, Chan MN, Chan CK. 2001. The hygroscopic properties of dicarboxylic and multifunctional acids: Measurements and UNIFAC predictions. Environ Sci Technol 35: 4495–4501.

88. Yttri KE, Dye C, Kiss G. 2007. Ambient aerosol concentrations of sugars and sugar-alcohols at four different sites in Norway. Atmos Chem Phys 7, 4267-4279.

89. Ariya PA, Sun J, Eltouny NA, Hudson ED, Hayes CT, Kos G. 2009. Physical and chemical characterization of bioaerosols – Implications for nucleation processes, Int Rev Phys Chem, 28:1, 1-32.

90. Goldberg LJ, Watkins HM, Boerke EE, Chatigny MA. 1958. The use of a rotating drum for the study of aerosols over extended periods of time. Am J Hyg 68:85-93.

91. Gruel RL, Reid CR, Allemann RT. 1987. The optimum rate of drum rotation for aerosol aging. J Aero Sci 18:17-22.

92. Asgharian B, Moss OR. 1992. Particle suspension in a rotating drum chamber when the influence and gravity are both significant. Aero Sci Tech 17:263-277.

93. Krumins V, Son E-K, Mainelis G, Fennell DE. 2008. Retention of inactivated bioaerosols and ethane in a rotating bioreactor constructed for bioaerosols activity studies. Clean 36:593-600.

94. Verrault D, Duchaine C, Marcoux-Voiselle M, Turgeon N, Roy CJ. 2014. Design of an environmentally controlled rotating chamber for bioaerosols aging studies. Inhal Toxicol 26:554-558.

95. Druett HA. 1971. A safe method of exposing microthreads in the open air. J Appl Microbiol 34:655-658.

96. May KR, Druett HA. 1968. A microthread technique for studying the viability of microbes in a simulated airborne state. J Gen Microbiol 51:353-366.

97. Handley BA, Roe JM. 1994. An alternative microthread for the study of airborne survival of bacteria outdoors. J Appl Bacteriol 77:504-508.

98. Smither SJ, Piercy TJ, Eastaugh L, Steward JA, Lever MS. 2011. An alternative method of measuring aerosol survival using spiders' webs and its use for filoviruses. J Virol Meth 177:123-127.

99. Adams DJ, Spendlove JC, Spendlove RS, Barnett BB. 1982. Aerosol stability of infectious and potentially infectious reovirus particles. Appl Environ Microbiol 44:903-908.

100. Sattar SA, Ijaz MK, Johnson-Lussenburg CM, Springthorpe CS. 1984. Effect of relative humidity on the airborne survival of rotavirus SA11. Appl Environ Microbiol 47:879-881.

101. Deguillaume L, Leriche M, Amato P, Arita PA, Delort A-M, Pöschl U, Chaumerliac N, Bauer H, Flossmann AI, Morris CE. 2008. Microbiology and atmospheric processes: chemical interactions of primary biological aerosols. Biogeosciences 5:1073-1084.

102. Estillore AD, Trueblood JV, Grassian VH. 2016. Atmospheric chemistry of bioaerosols: heterogeneous and multiphase reactions with atmospheric oxidants and other trace gases. Chem Sci 7:6604-6616.

103. Cox CS. 1989. Airborne bacteria and viruses. Sci Prog Oxford 73:469-500.

104. Einarsson H, Snygg BG, Eriksson G. 1983. Inhibition of bacterial growth by maillard reaction products. J Agric Food Chem 31:1043-1047.

746 105. Hess GE. 1965. Effects of oxygen on aerosolized *Serratia marcescens*. Appl
747 Microbiol 13:781-787.
748 106. Benbough JE. 1967. Death mechanisms in airborne *Escherichia coli*. J Gen
749 Microbiol 47:325-333.
750 107. Cox CS, Baldwin F. 1967. The toxic effect of oxygen upon the aerosol survival of
751 *Escherichia coli* B. J Gen Microbiol 49:115-117.
752 108. Hawkins LN, Lemire AN, Galloway MM, Corrigan AL, Turley JJ., Espelien BM, De
753 Haan DO. 2016. Maillard chemistry in clouds and aqueous aerosol as a source of
754 atmospheric humic-like substances, Environ Sci Technol 50:7443–7452.
755 109. Santarpia JL, Pan Y-L, Hill SC, Baker N, Cottrell B, McKee L, Ratnesar-Shumate
756 S, Pinnick RG. 2012. Changes in fluorescence spectra of bioaerosols exposed to
757 ozone in a laboratory reaction chamber to simulate atmospheric aging. Optics
758 Express 20:29867-29881.
759 110. Pan Y-L, Santarpia JL, Ratnesar-Shumate S, Corson E, Eshbaugh J, Hill SC,
760 Williamson CC, Coleman M, Bare C, Kinahan S. 2014. Effects of ozone and relative
761 humidity on fluorescence spectra of octapeptide bioaerosol particles. J Quant Spect
762 Rad Trans 538-550.
763 111. Ratnesar-Shumate S, Pan Y-L, Hill SC, Kinahan S, Corson E, Eshbaugh J,
764 Santarpia JL. 2015. Fluorescence spectra and biological activity of aerosolized
765 *Bacillus* spores and MS2 bacteriophage exposed to ozone at different relative
766 humidities in a rotating drum. J Quant Spect Rad Trans 153:13-28.
767 112. Cox CS. 1970. Aerosol survival of *Escerichia coli* B disseminated from the dry
768 state. Appl Microbiol 19:604-607.

769 113. Cox CS. 1966b. The survival of *Escherichia coli* in nitrogen atmospheres under
770 changing conditions of relative humidity. J Gen Microbiol 45:283-288.
771 114. Yang W, Marr, LC. 2012. Mechanisms by which ambient humidity may affect
772 viruses in aerosols. Appl Environ Microbiol 78:6781-6788.
773 115. Beebe JM. 1959. Stability of disseminated aerosols of *Pasteurella tularensis*
774 subjected to simulated solar radiations at various humidities. J Bacteriol 78:18-24.
775 116. Tong Y, Lighthart B. 1997. Solar radiation has a lethal effect on natural
776 populations of culturable outdoor atmospheric bacteria. Atmos Environ 31:897-900.
777 117. Tong Y, Lighthart B. 1998. Effect of simulated solar radiation on mixed outdoor
778 atmospheric bacterial populations. FEMS Microbiol Ecol 26:311-316.
779 118. Berendt RF, Dorsey EL. 1971. Effect of simulated solar radiation and sodium
780 fluorescein on the recovery of Venezuelan Equine Encephalomyelitis virus from
781 aerosols. Appl Microbiol 21:447-450
782 119. Gray LJ, Beer J, Geller M, Haigh JD, Lockwood M, Matthes K, Cubasch U,
783 Fleitmann D, Harrison G, Hood L, Luterbacher J, Meehl GA, Shindell D, van Geel B,
784 White W. 2010. Solar influences on climate. Rev Geophys 48:1-53.
785 120. Rastogi RP, Richa, Kumar A, Tyagi MB, Sinha RP. 2010. Molecular mechanisms
786 of ultraviolet radiation-induced DNA damage and repair. J Nucleic Acids
787 doi:10.4061/2010/592980.
788 121. Santos AL, Gomes NC, Henriques I, Almeida A, Correia A, Cunha A. 2012.
789 Contribution of reactive oxygen species to UV-B induced damage in bacteria. J
790 Photochem Photobiol B 117:40-46.

122. de Mik G, de Groot I. 1977. Mechanisms of inactivation of bacteriophage ϕX174 and its DNA in aerosols by ozone and ozonized cyclohexene. J Hyg – Camb 78:199-211.

123. Dark FA, Nash T. 1970. Comparative toxicity of various ozonized olefins to bacteria suspended in air. J Hyg – Camb 68:245-252.

124. Hood AM. 1971. An indoor system for the study of biological aerosols in open air conditions. J Hyg – Camb 69:607-617.

125. Donaldson AI, Ferris NP. 1975. The survival of foot-and-mouth disease virus in open air conditions. J Hyg (Lond) 74:409-416.

126. Lighthart B. 1973. Survival of airborne bacteria in a high urban concentration of carbon monoxide. Appl Microbiol 25:86-91.

127. Lighthart B, Hiatt VE, Rossano AT. 1971. The survival of airborne *Serratia marcescens* in urban concentrations of sulphur dioxide. J Air Pollut Cont Assoc 21:639-642.

128. Ehrlich R, Miller S. 1972. Effect of NO_2 on airborne Venezuelan Equine Encephalomyelitis virus. Appl Microbiol 23:481-484.

129. Hood AM. 2009. The effect of open-air factors on the virulence and viability of airborne *Francisella tularensis*. Epidemiol Infect 137:753-761.

130. Benbough JE, Hood AM. 1971. Viricidal activity of open air. J Hyg Camb 69:619-626.

131. de Mik G, de Groot I. 1978. Breaks induced in the deoxyribonucleic acid of aerosolized *Escherichia coli* by ozonized cyclohexene. Appl Environ Microbiol 35:6-10.

814 132. Anderson JD. 1966. Biochemical studies of lethal processes in aerosols of
815 *Escherichia coli*. J Gen Microbiol 45:303-313.
816 133. Cox CS. 1969. The cause of loss of viability of airborne *Escherichia coli* K12. J
817 Gen Microbiol 57:77-80.
818 134. Csonka LN. 1989. Physiological and genetic responses of bacteria to osmotic
819 stress. Microbiol Rev 53:121-147.
820 135. Sleator RD, Hill C. 2002. Bacterial osmoadaptation: the role of osmolytes in
821 bacterial stress and virulence. FEMS Microbiol Rev 26:49-71.
822 136. Wood JM. 2015. Bacterial responses to osmotic challenges. J Gen Physiol
823 145:381-388.
824 137. Anderson JD, Dark FA, Peto S. 1968. Studies on the effects of aerosolization on
825 the rates of efflux of ions from populations of *Escherichia coli* strain B. J Gen
826 Microbiol 46:95-105.
827 138. Gruzdev N, McClelland M, Porwollik S, Ofaim S, Pinto R, Saldinger-Sela S. 2012.
828 Global transcriptional analysis of dehydrated *Salmonella enterica* serovar
829 Typhimurium. Appl Environ Microbiol 78:7866-7875.
830 139. Kocharunchitt C, King T, Gobius K, Bowman JP, Ross T. 2014. Global genome
831 response of *Escherichia coli* O157:H7 Sakai during dynamic changes in growth
832 kinetics induced by an abrupt downshift in water activity. PLOS One 9:e90422
833 140. Fung KH, Tang IN. 2000. Chemical characterization of aerosol particles by laser
834 raman spectroscopy, p 177-195. *In* Spurny KR (ed), Aerosol Chemical Processes in
835 the Environment. CRC Press LLC, Florida.

836 141. Rubel GO. 2000. Novel applications of the electrodynamic levitator for the study
837 of aerosol chemical processes, p 197-211. *In* Spurny KR (ed), Aerosol Chemical
838 Processes in the Environment. CRC Press LLC, Florida.

839 142. Reid JP, Meresman H, Mitchem L, Symes R. 2007. Spectroscopic studies of the
840 size and composition of single aerosol droplets. Int Rev Phys Chem 26:139–192.

841 143. Reid JP. 2009. Particle levitation and laboratory scattering. J Quant Spectrosc
842 Radiat Transf 110:1293–1306.

843 144. Ault AP, Zhao D, Ebben CJ, Tauber MJ, Geiger FM, Prather KA, Grassian VH.
844 2013. Raman microspectroscopy and vibrational sum frequency generation
845 spectroscopy as probes of the bulk and surface compositions of size-resolved sea
846 spray aerosol particles. Phys Chem Chem Phys 15:6206-6214.

847 145. Craig RL, Bondy AL, Ault AP. 2015. Surface enhanced raman spectroscopy
848 enables observations of previously undetectable secondary organic aerosol
849 components at the individual particle level. Anal Chem 87:7510-7514.

850 146. Xie C, Li Y-Q. 2003. Confocal micro-Raman spectroscopy of single biological
851 cells using optical trapping and shifted excitation difference techniques. J Appl Phys
852 93:2982-2986.

853 147. Kalasinsky KS, Hadfield T, Shea AA, Kalasinsky VF, Nelson MP, Neiss J, Drauch
854 AJ, Vanni GS, Treado PJ. 2007. Raman chemical imaging spectroscopy reagentless
855 detection and identification of pathogens: signature development and evaluation.
856 Anal Chem 79:2658-2673.

857 148. Meisel S, Stöckel S, Elschner M, Melzer F, Rösch P, Popp J. 2012. Raman
858 spectroscopy as a potential tool for detection of *Brucella* spp. in milk. Appl Environ
859 Microbiol 78:5575-5583.
860 149. Zu TNK, Athamneh AIM, Wallace RS, Collakova E, Senger RS. 2014. Near real-
861 time analysis of the phenotypic response of *Escherichia coli* to 1-butanol exposure
862 using Raman spectroscopy. J Bacteriol 196:3983-3991.
863 150. Sengupta A, Laucks ML, Dildine N, Drapala E, Davis EJ. 2005. Bioaerosol
864 characterization by surface-enhanced Raman spectroscopy (SERS). Aero Sci
865 36:651-664.
866 151. Sengupta A, Brar N, Davis EJ. 2007. Bioaerosol detection and characterization
867 by surface-enhanced Raman spectroscopy. J Colloid Interface Sci 309:36-43.
868 152. Wang C, Pan Y-L, Hill SC, Redding B. 2015. Photophoretic trapping-Raman
869 spectroscopy for single pollens and fungal spores trapped in air. J Quant Spect Rad
870 Trans 153:4-12.
871 153. Cruz-Sanchez TM, Haddrell AE, Hackett TL, Singhera GK, Marchant D, Lekivetz
872 R, Meredith A, Horne D, Knight DA, van Eeden SF, Bai TR, Hegele RG, Dorscheid
873 DR, Agnes GR. 2013. Formation of a stable mimic of ambient particulate matter
874 containing viable infectious respiratory syncytial virus and its dry-deposition directly
875 onto cell cultures. Anal Chem 85:898-906.
876 154. Hingston PA, Piercey MJ, Hansen LT. 2015. Genes associated with desiccation
877 and osmotic stress in *Listeria monocytogenes* as revealed by insertional
878 mutagenesis. Appl Environ Microbiol 81:5350-5362.
879 155. Wang D, Bodovitz S. 2010. Single cell analysis: the new frontier in 'Omics'.
880 Trends Biotechnol 28:281-290.

156. Heinemann M, Zenobi R. 2011. Single cell metabolomics. Curr Opin Biotechnol 22:26-31.

157. Blainey PC. 2013. The future is now: single-cell genomics of bacteria and archaea. FEMS Microbiol Rev 37:1-29.

158. Tsioris K, Torres AJ, Douce TB, Love JC. 2014. A new toolbox for assessing single cells. Ann Rev Chem Biomol Eng 5:455-477.

159. Kalisky T, Quake SR. 2011. Single-cell genomics. Nat Meth 8:311-314.

160. Hall RJ, Leblanc-Maridor M, Wang J, Ren X, Moore NE, Brooks CR, Peacey M, Douwes J, McLean D. (2013). Metagenomic detection of viruses in animal slaughterhouses. PLoS One 8:e72226.

161. Be NA, Thissen JB, Fofanov VY, Allen JE, Rojas M, Golovko G, Fofanov Y, Koshinsky H, Jiang CJ. 2015. Metagenomic analysis of the airborne environment in urban spaces. Microb Ecol 69:346-355.

162. May KR. 1973. The Collison nebulizer: description, performance and application. Aero Sci 4:235-243.

163. Cox CS, Goldberg LJ. 1972. Aerosol survival of *Pasteurella tularensis* and the influence of relative humidity. Appl Microbiol 23:1-3.

164. Ehrlich R, Miller S. 1973. Survival of airborne *Pasteurella tularensis* at different atmospheric temperatures. Appl Microbiol 25:369-372.

165. Cox CS, Bondurant MC, Hatch MT. 1971. Effects of oxygen on aerosol survival of radiation sensitive and resistant strains of *Escherichia coli* B. J Hyg – Camb 69:661-672.

166. Woo M-H, Grippin A, Anwar D, Smith T, Wu C-Y, Wander JD. 2012. Effects of relative humidity and spraying medium on UV decontamination of filters loaded with viral aerosols. Appl Environ Microbiol 78:5781-5787.

167. Chen BT, John W. 2001. Instrument Calibration. *In* Baron PA, Willeke K (eds). Aerosol Measurement: Principles, Techniques, and Applications. Wiley, New York.

168. Young HW, Larson EW, Dominik JW. 1974. Modified spinning top homogeneous spray apparatus for use in experimental respiratory disease studies. Appl Microbiol 28:929-934.

169. Thomas RJ, Webber D, Sellors W, Collinge A, Stagg AJ, Bailey SC, Jayasekera PN, Taylor RR, Eley SE, Titball RW. 2008. Characterization and deposition of respirable large- and small-particle bioaerosols. Appl Environ Microbiol 74:6437-6443.

170. Grinshpun SA, Willeke K, Ulevicius V, Juozaitis A, Terzieva S, Donnelly J, Stelma GN, Brenner KP. 1997. Effect of impaction, bounce and reaerosolization on the collection efficiency of impingers. Aero Sci Technol 26:326-342.

171. Li C-S, Hao M-L, Lin W-H, Chang C-W, Wang C-S. 1999. Evaluation of aerosol samplers for bacterial microorganisms. Aero Sci Technol 30:100-108.

172. Lin X, Reponen TA, Willeke K, Grinshpun SA, Foarde KK, Ensor DS. 1999. Long-term sampling of airborne bacteria and fungi into a non-evaporating liquid. Atmos Environ 33:4291-4298.

173. Lin X, Reponen T, Willeke K, Wang Z, Grinshpun SA, Trunov M. 2000. Survival of airborne microorganisms during swirling aerosol collection. Aero Sci Technol 32:184-196.

926 174. Riemenschneider L, Woo W-H, Wu C-Y, Lundgren D, Wander J, Lee J-H & Li H-
927 W. 2009. Characterization of reaerosolization from impingers in an effort to improve
928 airborne virus sampling. J Appl Microbiol 108:315-324.
929 175. May KR, Harper GJ. 1957. The efficiency of various liquid impinger samplers in
930 bacterial aerosols. Brit J Industr Med 14:287-297.
931 176. Kesavan J, Schepers D, McFarland AR. 2010. Sampling and retention
932 efficiencies of batch-type liquid-based bioaerosols samplers. Aero Sci Technol
933 44:817-829.
934 177. Yu KP, Chen Y-P, Gong J-Y, Cen Y-C, Cheng C-C. 2016. Improving the
935 collection efficiency of the liquid impinger for ultrafine particles and viral aerosols by
936 applying granular bed filtration. J Aero Sci 101:133-143.
937 178. Zheng Y, Yao M. 2017. Liquid impinger Biosampler's performance for size-
938 resolved viable bioaerosol particles. J Aero Sci 106:34-42.
939 179. Andersen AA. 1958. New sampler for the collection, sizing and enumeration of
940 viable airborne particles. J Bacteriol 76:471-484.
941 180. May KR. 1975. An "ultimate" cascade impactor for aerosol assessment. J Aero
942 Sci 6:413-419.
943 181. Li C-S, Lin Y-C. 1999. Sampling performance of impactors for bacterial
944 bioaerosols. Aero Sci Technol 30:280-287.
945 182. Mainelis G, Willeke K, Adhikari A, Reponen T, Grinshpun SA 2002. Design and
946 collection efficiency of a new electrostatic precipitator for bioaerosol collection. Aero
947 Sci Technol 36:1073-1085.
948 183. Cheng YS. 2014. Mechanisms of pharmaceutical aerosol deposition in the
949 respiratory tract. AAPS Pharm Sci Tech 15:630-640.

184. Rabey F, Janssen RJ, Kelley LM. 1969. Stability of St. Louis Encephalitis virus in the airborne state. Appl Microbiol 18:880-882.

185. Hood AM. 1974. Open-air factor in enclosed systems. J Hyg 72:53-60.

186. Bailey R, Fielding L, Young A, Griffith C. 2007. Effect of ozone and open air factor against aerosolized *Micrococcus luteus*. J Food Prot 70: 2679-2773.

187. Walter MV, Marthi B, Fieland VP, Ganio LM. 1990. Effect of aerosolization on subsequent bacterial survival. Appl Environ Microbiol 56:3468-3472.

188. Marthi B, Fieland VP, Walter M, Seidler RJ. 1990. Survival of bacteria during aerosolization. Appl Environ Microbiol 56:3463-3467.

189. Cox CS. 1968b. The aerosol survival and cause of death of *Escherichia coli* K12. J Gen Microbiol 54:169-175.

190. Hatch MT, Dimmick RL. 1966. Physiological responses of airborne bacteria to shifts in relative humidity. Bacteriol Rev 30:597-602.

191. Ehrlich R, Miller S. 1971. Effect of relative humidity and temperature on airborne Venezuelan Equine Encephalitis virus. Appl Microbiol 22:194-199.

192. Wathes CM, Howard K, Webster AJF. 1986. The survival of *Escherichia coli* in an aerosol at air temperatures of 15 and 30 °C and a range of humidities. J Hyg Camb 97:489-496.

193. Thompson K-A, Bennett AM, Walker JT. 2011. Aerosol survival of *Staphylococcus epidermidis*. J Hosp Infect 78:216-220.

194. Ehrlich R, Miller S, Walker RL. 1970. Relationship between atmospheric temperature and survival of airborne bacteria. Appl Microbiol 19:245-249.

195. Benbough JE. 1969. Factors affecting the toxicity of oxygen towards airborne coliform bacteria. J Gen Microbiol 56:241-250.

196. Cox CS, Derr JS, Flurie EG, Roderick RC. 1970. Experimental technique for studying aerosols of lyophilized bacteria. Appl Microbiol 20:927-934.

197. Schlamm NA. 1960. Detection of viability in aged or injured *Pasteurella tularensis*. J Bacteriol 91:2180-2184.

198. Sawyer WD, Jemski JV, Hogge AL, Eigelsbach HT, Wolfe EK, Dangerfield HG, Gochenour WS, Crozier D. 1966. Effect of aerosol age on the infectivity of airborne *Pasteurella tularensis* for *Mucaca mulatta* and man. J Bacteriol 91:2180-2184.

199. Hood AM. 1961. Infectivity of *Pasteurella tularensis* clouds. J Hyg 59:497-504.

.

993 TABLE 1 Methods used to generate and sample microbial aerosols useful for aerosol fate and inhalational infection research.

AEM.00134-18

Mechanism	Apparatus examples	Description	References(s)
Aerosol generation			
Reflux nebulization (1-, 3-, 6-jet versions commonly used)	Collison nebulizer, Wells atomizer, TSI 9302, FK-8 aerosol gun, Aeroneb Lab	• Refluxing two-fluid atomizer operating by venturi effect and wall impaction. Liquid recirculation occurs every 6 seconds in the 3-jet version (135). • Increased jet numbers increase the rate of aerosol generation and recirculation. Reservoir evaporation occurs over time causing concentration effects. • Generally used for liquids, although the Wells atomizer was used for dry powders. Particle sizes are small, 0.7–2.2 μm. • Forces associated with reflux nebulization can cause deagglomeration of aggregates causing an observed increase in bacterial concentration in the spray suspension.	14, 16, 20, 23-25, 79, 80, 99, 122, 162-167
Non-reflux nebulization	Single-pass aerosolizer	• Atomisation as above minus wall impaction and recirculation	24
Aerosol bubbling	SLAG[b] and variants	• Liquid dripped onto a membrane is broken into droplets by air flow through the membrane. • Droplets burst due to increased pressure gradient between the inside and outside	16, 24, 26

		of the device generating small aerosol particles.	
Centrifugal atomization	Spinning top aerosol generator	• Centrifugal forces moves liquid applied to a rotating disc towards the edges producing ligands that break into droplets	168
Flow-focussing	FFAG[c], C-Flow nebulizer	• Liquid flows through an orifice forming microjets that break-up into particles by aerodynamic suction of an accelerated air stream. • Good monodispersity of droplets can be achieved.	20, 24, 169
Aerosol sampling			
Impingement	Impingers[d] (AGI-4, AGI-30, Model 7541 AGI); SKC biosampler	• Aerosol accelerates through critical orifice causing inertial impaction into liquid. • Efficiency is affected by physical parameters (e.g. sampling flow rate, nozzle number and angle, distance of nozzle from the liquid, solution type and volume, particle bounce, prolonged sampling time (liquid evaporation, increased damage) and binding of microorganisms to the collection vessel wall. • Reaerosolization can occur due to liquid bubbling. • Addition of glass beads can increase virus collection efficiency • SKC biosampler possesses three angled nozzles creating a gentler swirling	17, 18, 21, 22, 170-178

		motion of the bioaerosol during collection. • AGI-30 impaction velocity reaches 265 $m \cdot s^{-1}$; much reduced in other samplers.	
Impaction	Single or multistage impactors: Andersen, Mercer, Ultimate, MAS-100, Burkard	• Operate at constant flow rates, with air flowing through an orifice causing inertial impaction of particles too large to remain entrained in the air flow; size fractionation possible. • Collection can be onto a range of different substrates (e.g. agar plates, gelatin coated slides or filters). • Substrate choice can affect collection efficiency due to effects on microbial viability and particle bounce. • In the Burkard and 6th stage of the Andersen impactors, impaction velocities reach 12 and 24 $m \cdot s^{-1}$ respectively.	21, 22, 47, 179-181
Filtration/ impaction	Gelatin filter, nitrocellulose, polycarbonate	• Greater physical sampling efficiencies. Biological sampling efficiency may be lower due to sensitivity of the collected microorganisms to air drawn past the filter. • Elution of material from the filter surface (e.g. vortexing, shaking, solution volume and type) can influence efficiency.	21, 22, 48, 49

Direct capture	Microthreads	• Particles collected onto fine microthreads (e.g. spider silk, glue thread) wound on to a frame.	78, 96-98, 123-125
Cyclonic separation	NIOSH cyclonic biosampler	• Air flow drawn into a cylindrical container is rotated causing larger particles to deposit and collect on the walls by centrifugal forces.	25, 35
Electrostatic precipitation	Ionizers e.g. AS150; Model 3100 aerosol sampler	• Airborne particles electrically charged and subjected to electric field causing gentle deposition velocity onto collection substrate. • Bioefficiency for spores greater than for Gram-negative bacteria. • Impaction velocities reach 0.01 - 1 $m \cdot s^{-1}$.	30, 36, 182
Animal inhalation	Rodent, primates	• Aerosol particles regionally deposit due to inertial impaction, sedimentation, diffusion, interception and electrostatic effects in the respiratory tract. • Deposition is a function of airway geometry and particle properties (e.g. size, shape, density, hygroscopicity).	183

994

995 [a] = Note that the list is merely representative and not exhaustive. Researchers are recommended to conduct rigorous validation of
996 the aerosol experimental system for each individual micro-organism tested; [b] = sparging liquid aerosol generator; [c] = flow focussing
997 aerosol generator; [d] = all-glass impinger

998 TABLE 2 Examples of experimental techniques used to study fate of microorganisms
999 in aerosol

Device	Mechanism	Aerosol state[a]	Outdoor use?	References(s)
Rotating drum	Rotational speed of drum prevents aerosol from settling for period of time dependent on particle size	Dynamic	N	34, 82, 83, 93, 94, 99, 111, 126, 163, 184
Microthread	Aerosol captured on spider microthreads or glue fibres wound around a metal frame that can be slotted into an exposure apparatus.	Captured	Y	78, 96-98, 123-125, 130
Sphere	Steel sphere with mixing fans	Dynamic	N	124, 185
Aerosol chamber	Large chambers with mixing fans	Dynamic	N	186
Greenhouse	No mixing fan	Dynamic	Y	187,188

1000 [a] = Dynamic refers to particles maintained as a buoyant aerosol, whilst captured
1001 refers to aerosol particles immobilised on a substrate.

1002

1003

1004 TABLE 3 Atmospheric, environmental and microbial factors affecting survival and
1005 infectivity in airborne microorganisms

Factor	Description	References(s)[a]
Relative humidity (RH)	Generally studies range from 20 to 90% RH	41, 45, 76, 80, 82, 99, 100, 113, 115, 163, 184, 189-193
Temperature	Wide ranges studied from sub-zero to 50 °C	80, 164, 191, 192, 194
Solar radiation	Variability in spectra examined but inclusive of UV-A and UV-B wavelengths	46, 78, 115-118, 188
Oxygen	Generation of ROS[b] during aerosol transport	44, 105-107, 165, 195
Ozone	Reactive with pollutant gases and pinenes	122, 186
Pollutant gases 'Open air factor'	CO, SO_2, NO_2, ethene, cyclohexene SOAs[c] (e.g. alkenes, turpenes[d])	31, 93, 122-126, 127-131, 185
Wet / dry preparation	Droplets or dried particles	76, 112, 163, 189, 196
Growth phase	Exponential, stationary	31, 165
Particle size	Microbial aggregates have greater survival than single microorganisms	31, 78, 130, 195
Aerosol age	Infectivity decreased prior to culturability with extended time in aerosol	197-199

1006 [a] = reference list is reflective and not exhaustive; [b] = reactive oxygen species; [c] =
1007 secondary organic aerosol; [d] = turpenes are volatile cyclic unsaturated hydrocarbon
1008 molecules released by plants